DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

ADVANDX, INC.
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DIRECTOR RA/CA
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October 10, 2014

Re: K140619
Trade/Device Name: mecA XpressFISH
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial susceptibility test powder
Regulatory Class: II
Product Code: MYI
Dated: September 8, 2014
Received: September 9, 2014

Dear Mr. Eisenwinter:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
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and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K140619

Device Name
mecA XpressFISH

Indications for Use *(Describe)*
mecA XpressFISH® is a qualitative nucleic acid fluorescence in situ hybridization assay intended for the detection of mecA mRNA on smears from blood cultures that are positive for Staphylococcus aureus by the Staphylococcus QuickFISH™ BC assay.

The mecA XpressFISH® assay is indicated for use in conjunction with other laboratory tests and clinical data available to the clinician as an aid in the detection of mecA mRNA from methicillin-resistant S. aureus (MRSA) from patient positive blood cultures. The mecA XpressFISH® assay is not intended to monitor treatment for MRSA infections or for use with mixed cultures including those containing both S. aureus and coagulase negative staphylococci.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, epidemiological typing, and/or differentiation of mixed growth.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

510(k) Summary

510(k) Number: K140619

Applicant

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Establishment Registration Number

3004080598

Manufacturer Establishment Number:

3003994627

Device Trade Name:

mecA XpressFISH (formerly *mecA* PNA FISH)

Device Common Name:

System, Test, Genotypic Detection, Resistant Markers, *Staphylococcus* Colonies

Device Classification:

AdvanDx *mecA XpressFISH* is a Class II device and is classified by FDA under 21 CFR §866.1640, Antimicrobial Susceptibility Test Powder. The Product Code is MYI.

Intended Use and Indications For Use

*mecA Xpress*FISH® is a qualitative nucleic acid fluorescence *in situ* hybridization assay intended for the detection of *mecA* mRNA on smears from blood cultures that are positive for *Staphylococcus aureus* by the *Staphylococcus Quick*FISH™ BC assay.

The *mecA Xpress*FISH® assay is indicated for use in conjunction with other laboratory tests and clinical data available to the clinician as an aid in the detection of *mecA* mRNA from methicillin-resistant S. aureus (MRSA) from patient positive blood cultures. The *mecA Xpress*FISH® assay is not intended to monitor treatment for MRSA infections or for use with mixed cultures including those containing both *S. aureus* and coagulase negative staphylococci.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, epidemiological typing, and/or differentiation of mixed growth.

Special conditions for use statement(s)

For Prescription Use only.

Special instrument requirements

Fluorescence microscope with appropriate filter and light source.

Device Description

The *mecA Xpress*FISH assay is a qualitative fluorescence in situ hybridization (FISH) assay which utilizes peptide nucleic acid (PNA) probes hybridizing to *mecA* messenger RNA (mRNA) on smears from blood cultures containing *Staphylococcus aureus* (SA). After the positive blood culture bottle is confirmed to have SA by *Staphylococcus Quick*FISH BC, an aliquot of the blood culture is incubated with media containing cefoxitin to induce *mecA* mRNA expression prior to *mecA Xpress*FISH testing.

The assay consists of *mecA* mRNA inducing cefoxitin reagents, a fixation reagent, a hybridization reagent, a wash solution concentrate, coverslip mounting media and a microscope slide which has a sample well, and positive and negative control wells. Results are visualized and interpreted using fluorescence microscopy.

Substantial Equivalence:

For the identification of *mecA*-mediated methicillin (Oxacillin) resistance in *S. aureus,* the *mecA Xpress*FISH assay is substantially equivalent to the BinaxNOW PBP2a test (Alere, Maine, k090301), which detects the PBP2a protein responsible for methicillin resistance.

Comparison Tables of Similarities and Differences

Similarities		
Item	***mecA Xpress*FISH K140619**	**BinaxNOW PBP2a K090301**
Intended Use	*mecA Xpress*FISH® is a qualitative nucleic acid fluorescence *in situ* hybridization assay intended for the detection of *mecA* mRNA on smears from blood cultures that are positive for *Staphylococcus aureus* by the *Staphylococcus Quick*FISH™ BC assay. The *mecA Xpress*FISH® assay is indicated for use in conjunction with other laboratory tests and clinical data available to the clinician as an aid in the detection of *mecA* mRNA from methicillin-resistant S. aureus (MRSA) from patient positive blood cultures. The *mecA Xpress*FISH® assay is not intended to monitor treatment for MRSA infections or for use with mixed cultures including those containing both *S. aureus* and coagulase negative staphylococci. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, epidemiological typing, and/or differentiation of mixed growth.	The BinaxNOW® PBP2a Test is a qualitative, in vitro immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) present in methicillin-resistant Staphylococcus aureus (MRSA). The test is performed directly on blood culture samples positive for S. aureus. The BinaxNOW PBP2a Test is not intended to diagnose MRSA nor to guide or monitor treatment for MRSA infections. Subculturing positive blood cultures is necessary to recover organisms for susceptibility testing or epidemiological typing.
Sample Type	*S. aureus* positive blood cultures	Same
Test Principle	Detection of *mecA* gene expression	Same
Method of Result	Visual	Same

Similarities		
Item	***mecA Xpress*FISH K140619**	**BinaxNOW PBP2a K090301**
Interpretation		
Mode of Operation	Manual	Same

Differences		
Item	***mecA Xpress*FISH K140619**	**BinaxNOW PBP2a K090301**
Target Analyte	mecA mRNA	PBP2a protein
Test Method	Molecular detection of gene expression	Phenotypic detection of gene expression
Technology	*In situ* hybridization of fluorescently labeled peptide nucleic acid (PNA) probe	Immunochromatography with monoclonal antibodies that bind to the penicillin binding protein PBP2a
Sample Preparation	Cefoxitin induced expression of *mecA* expression	Recovery and washing of cells by centrifugation, followed by lysis
Controls	Integrated Positive and Negative Controls to monitor hybridization, washing and result interpretation	Separate preparation of test strip(s) for control organisms

Bench Testing

AdvanDx has conducted in-house bench testing of the *mecA Xpress*FISH assay. The types of testing included limit of detection, analytic inclusivity, cross-reactivity, microbial interference, interfering substances (including different blood culture media), cross-contamination, tolerance, and reproducibility. Additionally, tests were conducted to validate the timing of each step of the testing procedure as well as the storage conditions of prepared slides.

The results of this testing verify that for the detection of *mecA*-mediated methicillin resistance in *S. aureus* in blood cultures, *mecA Xpress*FISH is substantially equivalent to the BinaxNOW PBP2a predicate device.

Limit of Detection ("LoD")

Three different MRSA strains were used for the LoD study, MRSA ATCC 33591, ATCC 43300, and NRS383. Organisms were grown under simulated blood culture conditions.

The Limit of Detection was measured by serially diluting the MRSA strains in quadruplicate. The lowest readable concentration for each strain was re-tested in replicates of 20 and found to be $8.1x10^4 – 2.8x10^5$ CFU/mL. This LoD is comparable to that of *Staphylococcus Quick*FISH and other slide based microbiological tests such as Gram Stain.

Analytic Reactivity (Inclusivity) and Analytic Specificity (Exclusivity)

The panel of organisms used to evaluate the analytical inclusivity (reactivity) of the *mecA Xpress*FISH assay was comprised of 64 MRSA strains representing a broad spectrum of genotypes, clonal complexes, isolation dates, and locations. Of the 64 MRSA strains tested, only one, which is known not to carry the *mecA* gene, did not produce Green Positive results (*mecC* type).

For exclusivity (specificity), the panel included 30 strains of MSSA, 40 strains of other *Staphylococcus* species (i.e., coagulase-negative staphylococci [CoNS]), 36 strains of other gram-positive organisms, and 20 other clinically relevant species (i.e. gram-negatives and yeasts). Organisms were grown under simulated blood culture conditions. 29 of 30 MSSA produced Negative *mecA Xpress*FISH results, while 1 MSSA, known to be *mecA*+, gave a Green Positive result. Six of the 40 other CoNS produced Green Positive results while the remaining 34 were Negative. Of the 37 other Gram positive organisms, 36 tested Negative. *Lactococcus lactis* was Green Positive. Subsequent additional analytical testing also produced weak Green Positive fluorescence with *Micrococcus luteus*. One of the 19 other organisms tested (*Candida parapsilosis*) produced a Green Positive result, while the other 18 were Negative. Because *mecA Xpress*FISH is only indicated for use with blood cultures that contain Gram Positive Cocci in Clusters and which test Positive for *S. aureus* alone by *Staphylococcus Quick*FISH BC (i.e., pure cultures of *S. aureus*), the risk of False Positive results due to cross-reaction with other species is considered low.

Co-Infection

*mecA Xpress*FISH is intended to be used with blood cultures of *S. aureus* as determined by *Staphylococcus Quick*FISH BC and is not intended to be used with mixed cultures identified either by Gram stain or *Staphylococcus Quick*FISH BC. Because of the inherent analytic sensitivity of slide based microbial tests and the variable rate of growth of microbes, there exists a possibility that a blood culture which appears to be positive solely for *S. aureus* by Gram stain and *Staphylococcus Quick*FISH BC is in fact a mixed culture (*i.e.*, *S. aureus* and another species). In addition, should MRSA and MSSA occur together in the same blood culture, the culture would not be distinguishable as a mixed culture by either Gram stain or *Staphylococcus Quick*FISH BC. A microbial interference study was therefore conducted in order to

evaluate whether the presence of a non-MRSA organism would interfere with the detection of MRSA by *mecA Xpress*FISH.

Testing was performed with simulated blood cultures to determine the impact of mixed populations of MRSA and MSSA or MRSA and coagulase negative staphylococci on the *mecA Xpress*FISH assay. Two strains of MRSA were tested in the presence of different "co-infecting" species of methicillin-sensitive staphylococci: *S. aureus*, *S. epidermidis* and *S. simulans*. The strains of MRSA were both tested near the previously determined LoD of the assay, which is ~10^5 CFU/mL. The other organisms were all tested at about 10^8 CFU/mL. No False Positive results were obtained from the methicillin-sensitive staphylococci and no interference was observed in detection of MRSA in the presence of the co-infecting species.

Interfering Substances

Testing of potential interfering substances was performed at the concentrations shown in the following Table. Two MRSA strains, ATC 43300 (MRSA 1) and NRS 674 (MRSA 2), and two MSSA strains, ATCC 29213 (MSSA 1) and ATCC 11632 (MSSA 2), were tested. All tests with MRSA produced Positive results, although only weak fluorescence was observed in the presence of Linezolid. Testing of samples from patients treated with Linezolid could therefore produce False Negative results. This is reflected as a limitation in the *mecA Xpress*FISH Package Insert.

All MSSA strains showed Negative results.

Interference Testing Results

Substance	Concentra-tion	MRSA 1	MRSA 2	MSSA 1	MSSA 2
Amoxicillin	12 µg/mL	Pos.	Pos.	Neg.	Neg.
Clavulanate	3 µg/mL	Pos.	Pos.	Neg.	Neg.
Sulbactam	45 µg/mL	Pos.	Pos.	Neg.	Neg.
Ampicillin	120 µg/mL	Pos.	Pos.	Neg.	Neg.
Clindamycin	10 µg/mL	Pos.	Pos.	Neg.	Neg.
Daptomycin	130 µg/mL	Pos.	Pos.	Neg.	Neg.
Ibuprofen	50 µg/mL	Pos.	Pos.	Neg.	Neg.
Linezolid	38 µg/mL	Weak Pos.	Weak Pos.	Neg.	Neg.
Oxacillin	230 µg/mL	Pos.	Pos.	Neg.	Neg.
Vancomycin	50 µg/mL	Pos.	Pos.	Neg.	Neg.
Amoxicillin and Clavulanate	12 µg/mL 3 µg/mL	Pos.	Pos.	Neg.	Neg.
Sulbactam and Ampicillin	45 µg/mL 120 µg/mL	Pos.	Pos.	Neg.	Neg.
Bilirubin	150 µg/mL	Pos.	Pos.	Neg.	Neg.
Hemoglobin	~30 mg/mL	Pos.	Pos.	Neg.	Neg.
Triglycerides	3500 µg/mL	Pos.	Pos.	Neg.	Neg.
SPS	0.04%	Pos.	Pos.	Neg.	Neg.
Saponin	~0.2%	Pos.	Pos.	Neg.	Neg.

Compatible Blood Culture Media

An in-house study was conducted to assess the compatibility of *mecA Xpress*FISH with certain of the most commonly used blood culture media of the three predominant blood culture systems. Testing was performed using multiple clinical strains of *S. aureus* representing a wide range of geographic and genotypic strain diversity.

In all, there were nine different blood culture media bottles tested. *mecA Xpress*FISH testing for each MRSA produced a Positive result for all media types and each MSSA produced a Negative result for all media types, with an overall agreement of 100%. It is concluded that *mecA Xpress*FISH is compatible with the nine commonly utilized blood culture media that were tested.

Blood Culture Media Types Tested

Manufacturer	Blood Culture System	Type of Media Bottle	Catalog Number
Becton Dickinson	BD BACTEC™	Plus Aerobic/F	442192
		Plus Anaerobic/F*	442193
		Standard/10 Aerobic/F*	442260
		Standard Anaerobic/F*	442191
		Lytic/10 Anaerobic/F	442265
		Peds Plus*	442194
bioMérieux	BacT/ALERT®	SA-Standard Aerobic	259789
		SN-Standard Anaerobic*	259790
Thermo Scientific	VersaTREK	REDOX 1 aerobic*	7102-44

*Clinical performance with *mecA Xpress*FISH not established.

Cross-contamination (Wash Step)

According to the instructions for use, *mecA Xpress*FISH slides can be washed in batches of up to five slides following the hybridization step. This is the only step in the *mecA Xpress*FISH procedure where slides prepared from different samples (patients) are present and exposed together to a common reagent and where they might become cross-contaminated. AdvanDx conducted an in-house study with the *mecA Xpress*FISH assay to assess the potential for cross-contamination during the wash step leading to False Positive results.

A well characterized *mecA* positive MRSA strain (NRS 383) and a strain of MSSA (ATCC BAA 1718) were used for testing. For each run, a set of four MRSA slides and one MSSA slide was prepared from simulated blood cultures at $>10^8$ CFU/mL. Three sets of five slides were processed through the hybridization step according to the *mecA Xpress*FISH procedure and each slide set was then placed into a slide holder for the wash step. The placement of the MSSA slide relative to the four MRSA slides within the holder was varied between sets (i.e., the MSSA slide within a set was placed at a different middle or outside position relative to the four MRSA slides within the holder). The slide sets were each then washed according to the *mecA Xpress*FISH procedure, and the slides were mounted and examined by fluorescence microscopy. All MRSA samples were Green Positive (12/12, 100%) and all MSSA samples were Negative (3/3, 100%) with no Green Positive organisms found in the MSSA sample wells (or Internal Negative Control wells). The results verify that cross-contamination of slides is unlikely to occur when up to five slides are washed together.

Cefoxitin Concentration

It was established during the *mecA Xpress*FISH product development that a cefoxitin concentration of between 2.5 and 15 µg/mL in TSB is optimal for induction of the *mecA* gene within 40-50 min. at 35°C. AdvanDx conducted an in-house study to verify the functional range of cefoxitin concentration in TSB for the *mecA Xpress*FISH assay. A

panel of 20 diverse MRSA and three MSSA strains were grown under simulated blood culture conditions. The *mecA Xpress*FISH assay was then conducted with each sample where the cefoxitin concentration in TSB was set at three different levels: 2.5µg/mL, 6.1µg/mL and 15µg/mL. All 20 of the MRSA tested under the three cefoxitin concentrations for the induction step produced Positive *mecA Xpress*FISH test results (60/60; 100%) and all three MSSA produced Negative test results (9/9, 100%). The results verify that a cefoxitin concentration range of 2.5-15 µg/mL is suitable for induction of *mecA* mRNA expression.

Cefoxitin Induction Time Range

Exposure of MRSA to cefoxitin (or other methicillin-type drugs) induces the production of *mecA* mRNA. As part of the product development for *mecA Xpress*FISH, it was determined that 40 to 50 minutes of drug exposure is required to activate *mecA* gene transcription for MRSA strains that do not express (or only weakly express) *mecA* mRNA in the absence of drug exposure. In order to verify this time range, AdvanDx conducted an in-house study with the *mecA Xpress*FISH assay and a panel of well-characterized MRSA strains to assess test performance within the prescribed time range. A panel of 20 MRSA and three MSSA were tested at each of three time points: 40, 45 and 50 minutes. All 20 of the MRSA gave a Positive result for *mecA Xpress*FISH at each of the three time points tested (60/60, 100%). All three of the MSSA produced a Negative result for each time point (9/9, 100%). The results verify that a 40-50 min. induction time is suitable for induction of *mecA* mRNA.

Induction to Slide Preparation Interval

During the design of *mecA Xpress*FISH it was determined that users wished to have flexibility in the timing between completion of the cefoxitin induction step and preparation of smears from the induced culture. AdvanDx conducted an in-house study to assess whether a delay of up to one hour between induction and slide preparation would affect the performance of *mecA Xpress*FISH. A panel of 20 MRSA and 3 MSSA were grown under simulated blood culture conditions. Following the induction step, slides were prepared immediately and then at 30 and 60 minutes. Induced cultures were stored at room temperature prior to slide preparation. All 20 of the MRSA gave a Positive test result for *mecA Xpress*FISH at the three time points tested (60/60, 100%) and the three MSSA produced Negative results (9/9, 100%). The results verify that slides may be prepared for up to one hour after the completion of the induction step.

Fixed Slide Stability Testing

During the design of the *mecA Xpress*FISH assay it was determined that users wished to have flexibility in the timing between completion of the fixation step and the start of the hybridization step of the assay procedure. According to the instructions for use, fixed slides may be stored under the following conditions prior to the hybridization step:

- Store *mecA Xpress*FISH control slides in their original, individually sealed pouches at 2-8°C. Slides must be used immediately after breaking pouch seal. Do not use slides after the expiration date.

- Fixed *mecA* XpressFISH smears should be hybridized within 5 minutes following fixation and may be left on the slide warmer at 55 ± 1°C for up to 5 minutes before addition of the hybridization reagent (*mecA* PNA).
    - Prepared smears may be stored at room temperature for up to 1 hour prior to testing or may be stored at 2-8 °C for up to 24 hours before testing.
- Note: If smears were stored after fixation at 2-8°C or room temperature they must be placed on the slide warmer for approximately 5 minutes at 55 ± 1°C before adding the hybridization reagents.

AdvanDx conducted an in-house study of the *mecA Xpress*FISH assay to assess test performance within the prescribed fixed slide storage times and conditions. Multiple clinically relevant strains of *S. aureus* were tested under the described conditions. All MRSA tested under the various storage conditions and times produced Positive *mecA Xpress*FISH test results (158/158; 100%) and all three MSSA produced Negative test results (23/23, 100%). The results verify that following the fixation step, slides may be stored and processed according to the instructions for use, without affecting test performance.

Hybridization Time Range

During the design of the *mecA Xpress*FISH assay, it was determined that users would need to have flexibility in the duration of the hybridization step in order to accommodate batch processing of small numbers of slides and for other reasons. According to the instructions for use, the hybridization for *mecA Xpress*FISH can be performed within 10 to 20 minutes. AdvanDx conducted an in-house study to assess test performance within this prescribed time range. A panel of 20 MRSA and three MSSA were tested at each of three time points (10, 15, and 20 minutes of hybridization). The twenty MRSA gave a Positive test result for *mecA Xpress*FISH at the three time points tested (60/60, 100%) and the three MSSA produced Negative test results (9/9, 100%). The results verify that the hybridization time of between 10 and 20 minutes is suitable.

Slide Wash Time Range

According to the instructions for use, *mecA Xpress*FISH slides may be washed for between 10 to 20 minutes at 57°C following the hybridization step. An AdvanDx in-house study was conducted with the *mecA Xpress*FISH assay to assess test performance within this prescribed time range. A panel of 20 MRSA and three MSSA were tested at each of three time points: 10, 15, and 20 minutes. The twenty MRSA gave a Positive test result for *mecA Xpress*FISH at the three time points tested (60/60, 100%) and the three MSSA produced Negative test results (9/9, 100%). The results verify that a slide wash time of between 10 and 20 minutes is suitable.

Age of Blood Culture at *mecA Xpress*FISH Testing

Ideally in the clinical setting, *mecA Xpress*FISH should be performed as soon as possible after blood culture bottle positivity and the completion of *Staphylococcus Quick*FISH BC testing. However, the elapsed time from blood culture positivity to *mecA Xpress*FISH testing could vary from lab to lab, depending on such factors as laboratory staffing, reporting policies, and time of day at which bottles signal Positive. Hence, the age of a Positive blood culture bottle at the time of *mecA Xpress*FISH testing could be

as little as < 1 hour to greater than 48 hours. AdvanDx conducted an in-house bridging study to assess the performance of the test on blood cultures ranging from the time of bottle positivity to ~60 hours of age. A panel of 20 MRSA and three MSSA was used for testing and *mecA Xpress*FISH testing was performed at each of four time points: "Bottle Ring" (≤ 2 hrs.) , "Bottle Ring" + 6-12 hours, "Bottle Ring" + 24-28 hours and "Bottle Ring" + 54-64 hours. All of the 20 MRSA gave a Green Positive result for *mecA Xpress*FISH at all four time points tested (80/80, 100%). One strain, ATCC 700699, produced a weak (but detectable) Green Positive signal beyond the 6-12 hr. testing time. The three MSSA all produced Negative test results (12/12, 100%). The results verify that bottles may be tested up to 60 hours following positivity (Bottle Ring) without affecting the performance of *mecA Xpress*FISH.

Time to Viewing Results

According to the Package Insert, mounted slides prepared using the *mecA Xpress*FISH assay may be viewed for interpretation up to two hours after completion of the assay. An in-house study was conducted to assess the ability to interpret results within this prescribed time range. A panel of 20 MRSA and three MSSA was grown under simulated blood culture conditions and samples were processed with the *mecA Xpress*FISH assay as per the instructions for use. Slides were interpreted immediately following the procedure, one hour after the procedure, two hours after the procedure and 18-24 hours after the procedure. The 20 MRSA gave a Positive test result for *mecA Xpress*FISH at the four viewing time points (80/80, 100%) with some reduction in signal observed for some strains at the 18-24 hour time point. The three MSSA produced Negative test results for all time points (12/12, 100%). The results verify that viewing slides up to two hours following completion of the assay does not affect the performance of the *mecA Xpress*FISH assay.

Tolerance Testing

The critical steps of the *mecA Xpress*FISH assay were tested at different temperature ranges with a specified organism panel. The lower and upper inner limits of the required incubation temperatures, along with lower and upper outer limits of the incubation temperatures, were tested for the induction, fixation, hybridization, and wash steps. 20 MRSA, 3 MSSA, and 1 *M. luteus* were tested for these conditions.

All MRSA tested were Green Positive with *mecA Xpress*FISH, regardless of the tolerance conditions. All MSSA tested were Negative with *mecA Xpress*FISH, regardless of the tolerance conditions. Throughout the development of the *mecA Xpress*FISH assay, it has been noticed that *Micrococcus luteus* ATCC 49732 has exhibited sporadic green auto-fluorescence. *Micrococcus luteus* synthesizes weakly fluorescent chromophores as part of its normal physiology, as may be observed in the yellow pigmentation of colonies grown in TBS agar. Weak Green Positive results were observed with *M. luteus*, even with the low and high temperature conditions that are within tolerance. False Positive results may therefore be obtained with *M. luteus*. This is reflected as a Limitation in the *mecA Xpress*FISH Package Insert.

Reproducibility

A reproducibility study was performed with *mecA Xpress*FISH. Testing was performed in triplicate by two operators (per site) over five days.* The test panel consisted of two strains of MRSA and one of MSSA grown to two different concentrations. Operators were blinded to the identification of all samples. The results are presented below by site across 5 days and by five days across three sites.

Sample Matrix for *mecA Xpress*FISH Reproducibility

Strain	Strain ID	Set 1 Inoculum CFU/Bottle	Set 1 CFU/mL at Bottle Ring	Set 2 Inoculum CFU/Bottle	Set 2 CFU/mL at Bottle Ring + 8 Hours
MRSA	NRS 674	128	4.5×10^7	75	9.6×10^8
MRSA	ATCC 43300	59	3.4×10^7	64	1.1×10^8
MSSA	ATCC 29213	80	7.7×10^7	122	2.8×10^7

***mecA Xpress*FISH Reproducibility Results by Site for 5 Test Days**

	Site 1	Site 2	Site 3	Total
Positive Green Agreement	40/40	40/40	40/40	100% 120/120
Negative Agreement	20/20	20/20	20/20	100% 60/60
Total Agreement	100% 60/60	100% 60/60	100% 60/60	100% 180/180

***mecA Xpress*FISH Reproducibility Results by Day for 3 study Sites**

	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Positive Green Agreement	24/24	24/24	24/24	24/24	24/24	100% 120/120
Negative Agreement	12/12	12/12	12/12	12/12	12/12	100% 60/60
Total Agreement	100% 36/36	100% 36/36	100% 36/36	100% 36/36	100% 36/36	100% 180/180

* Note: This study was repeated because of several False Positive results obtained by one operator on one day at one site, the root cause for which could not be established. Only results from the repeat study are shown.

Clinical Studies

The performance of the *mecA Xpress*FISH assay compared to cefoxitin disk diffusion for identification of methicillin-resistant SA (MRSA) was determined in seven clinical sites (6 US and 1 ex-US). A total of 339 routine blood culture bottles that were determined to be positive for only *Staphylococcus aureus* by *Staphylococcus Quick*FISH BC were included in the study. The results showed 99.1% (336/339) agreement between *mecA Xpress*FISH and cefoxitin disk diffusion for identification of MRSA. The sensitivity and specificity were determined to be 98.7% and 99.5%,

respectively. The performance of *mecA Xpress*FISH versus cefoxitin disk diffusion is presented in the following Table:

_mecA Xpress_FISH Performance vs. Cefoxitin Disk Diffusion

	Cefoxitin Disk Diffusion Methicillin Resistant (≤ 21mm)	**Methicillin Susceptible (≥ 22mm)**	
***mecA Xpress*FISH Positive**	151	1[1]	**PPV** 99.3%(151/152) 95% CI (96.4-99.9)
***mecA Xpress*FISH Negative**	2[2]	185	**NPV** 98.9%(185/187) 95% CI 96.2-99.7
N=339	**Sensitivity** 98.7% (151/153) 95%CI (95.4-99.6)	**Specificity** 99.5% (185/186) 95% CI (97.0-99.9)	

[1]False Positive *mecA* (weak Green Positive); Negative upon repeat testing. Cefoxitin disk=28 mm.
[2]Two False Negative *mecA*: For one, repeat testing was Negative, and for the other it was Positive (weak Green Positive). Cefoxitin disk diffusion results were19mm and 11mm, respectively.

An analysis of the results of the clinical study by site and type of culture medium is presented in the table below.

Bottle Type	**Site**	**Methicillin Resistant (*mecA*+)**	**Methicillin Susceptible (*mecA*-)**	**Total (%; 95% CI)**
BACTEC Plus Aerobic/F	A	2/2	12/12	14/14 (100)
	B	18/19	17/17	35/36 (97.2)
	C	7/7	18/19	25/26 (96.2)
	D	7/7	14/14	21/21 (100)
	E	19/19	19/19	38/38 (100)
	Total (%; 95% CI)	**53/54 (98.1; 90.2-99.7)**	**80/81 (98.8; 93.3-99.8)**	**133/135 (98.5; 94.8-99.6)**
BACTEC Lytic/10 Anaerobic/F	A	7/7	10/10	17/17 (100)
	B	10/10	17/17	27/27 (100)
	C	10/10	14/14	24/24 (100)
	D	19/19	4/4	23/23 (100)
	E	13/14	13/13	26/27 (96.3)
	Total (%; 95% CI)	**59/60 (98.3; 91.1-99.7)**	**58/58 (100; 93.8-100)**	**117/118 (99.2; 95.4-99.9)**

Bottle Type	Site	Methicillin Resistant (*mecA*+)	Methicillin Susceptible (*mecA*-)	Total (%; 95% CI)
BACTEC Peds Plus	B	1/1	1/1	2/2 (100)
	C	2/2	5/5	7/7 (100)
	D	0	1/1	1/1 (100)
	Total (%; 95% CI)	**3/3 (100; 43.9-100)**	**7/7 (100; 64.6-100)**	**10/10 (100; 72.3-100)**
BACTEC Plus Anaerobic/F	**B [Total] (%; 95% CI)**	**7/7 (100; 64.6-100)**	**5/5 (100; 56.6-100)**	**12/12 (100; 75.8-100)**
BACTEC Standard/10 Aerobic/F	**D [Total] (%; 95% CI)**	**0**	**1/1 (100; 20.7-100)**	**1/1 (100; 20.7-100)**
BACTEC All Media	**Total (%; 95% CI)**	**122/124 (98.4; 94.3-99.6)**	**151/152 (99.3; 96.4-99.9)**	**273/276 (98.9; 96.9-99.6)**
BacT/ALERT SA Standard Aerobic	F	24/24	18/18	42/42 (100)
	G	1/1	8/8	9/9 (100)
	Total (%; 95% CI)	**25/25 (100; 86.7-100)**	**26/26 (100; 87.1-100)**	**51/51 (100; 93.0-100)**
BacT/ALERT SN Standard Anaerobic	F	4/4	2/2	6/6 (100)
	G	0	6/6	6/6 (100)
	Total (%; 95% CI)	**4/4 (100; 51.0-100)**	**8/8 (100; 67.6-100)**	**12/12 (100; 75.8-100)**
BacT/ALERT All Media	**Total (%; 95% CI)**	**29/29 (100; 88.3-100)**	**34/34 (100; 89.9-100)**	**63/63 (100; 94.2-100)**

Conclusion

It is concluded from the data presented above, that the identification of *mecA*-mediated methicillin resistance in *Staphylococcus aureus* by the *mecA Xpress*FISH assay from *S. aureus* positive blood culture bottles as identified by *Staphylococcus Quick*FISH BC is substantially equivalent to identification a previously cleared predicate method and does not raise new types of safety or effectiveness questions when used as labelled.